

34. The method of claim 33, wherein said endonuclease recognition site is selected from the group consisting of Class I I-endonuclease sites, Class II I-endonuclease sites, Class III I-endonuclease sites, Class IV I-endonuclease sites, and Class V I-endonuclease sites.

35. The method of claim 34, wherein said endonuclease recognition site is a Class I I-endonuclease site.

36. The method of claim 35, wherein said endonuclease recognition site is selected from the group consisting of I-SceI, I-SceIV, I-CsmI, and I-PanI sites.

37. The method of claim 36, wherein said endonuclease recognition site is an I-SceI site. --

REMARKS

Entry and consideration of this amendment is respectfully requested.

Claims 1-26 have been canceled. New claims 27-37 find support throughout the specification, for example on pages 20-21 and Fig.6. Accordingly, no new matter is entered by amendment.

Applicants submit herewith a Sequence Listing and have amended the specification to conform with the requirements of 37 C.F.R. §§ 1.821-1.825.

Applicants request the use of the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, now U.S. Patent No. 5,962,327, issued October 5, 1999.

08/417-226

LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, DC 20005  
202-408-4000

I hereby state that the contents of the paper copy of the Sequence Listing in this application and the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same.

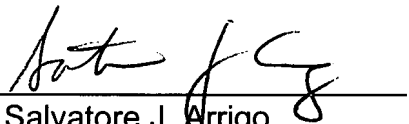
Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,  
GARRETT & DUNNER, L.L.P.

Dated: April 18, 2001

By: 

Salvatore J. Arrigo  
Reg. No. 46,063  
Tel: (202) 408-4000  
Fax: (202) 408-4400  
Email: arrigos@finnegan.com

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LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, DC 20005  
202-408-4000

B

Version with Markings to Show Changes MadeIn the Specification:

The paragraph beginning on line 4 of page 5 has been amended as follows:

Accordingly, this invention aids in fulfilling these needs in the art. Specifically, this invention relates to an isolated DNA encoding the enzyme I-SceI. The DNA has the following nucleotide sequence:

|      |     |     |     |     |     |     |                |     |     |     |     |     |      |     |     |     |     |     |     |     |      |
|------|-----|-----|-----|-----|-----|-----|----------------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|------|
|      | ATG | CAT | ATG | AAA | AAC | ATC | AAA            | AAA | AAC | CAG | GTA | ATG | 2670 |     |     |     |     |     |     |     |      |
|      | M   | H   | M   | K   | N   | I   | K              | K   | N   | Q   | V   | M   | 12   |     |     |     |     |     |     |     |      |
| 2671 | AAC | CTC | GGT | CCG | AAC | TCT | AAA            | CTG | CTG | AAA | GAA | TAC | AAA  | TCC | CAG | CTG | ATC | GAA | CTG | AAC | 2730 |
| 13   | N   | L   | G   | P   | N   | S   | K              | L   | L   | K   | E   | Y   | K    | S   | Q   | L   | I   | E   | L   | N   | 32   |
| 2731 | ATC | GAA | CAG | TTC | GAA | GCA | GGT            | ATC | GGT | CTG | ATC | CTG | GGT  | GAT | GCT | TAC | ATC | CGT | TCT | CGT | 2790 |
| 33   | I   | E   | Q   | F   | E   | A   | G              | I   | G   | L   | I   | L   | G    | D   | A   | Y   | I   | R   | S   | R   | 52   |
| 2791 | GAT | GAA | GGT | AAA | ACC | TAC | TGT            | ATG | CAG | TTC | GAG | TGG | AAA  | AAC | AAA | GCA | TAC | ATG | GAC | CAC | 2850 |
| 53   | D   | E   | G   | K   | T   | Y   | C              | M   | Q   | F   | E   | W   | K    | N   | K   | A   | Y   | M   | D   | H   | 72   |
| 2851 | GTA | TGT | CTG | CTG | TAC | GAT | CAG            | TGG | GTA | CTG | TCC | CCG | CCG  | CAC | AAA | AAA | GAA | CGT | GTT | AAC | 2910 |
| 73   | V   | C   | L   | L   | Y   | D   | Q              | W   | V   | L   | S   | P   | P    | H   | K   | K   | E   | R   | V   | N   | 92   |
| 2911 | CAC | CTG | GGT | AAC | CTG | GTA | ATC            | ACC | TGG | GGC | GCC | CAG | ACT  | TTC | AAA | CAC | CAA | GCT | TTC | AAC | 2770 |
| 93   | H   | L   | G   | N   | L   | V   | I              | T   | W   | G   | A   | Q   | T    | F   | K   | H   | Q   | A   | F   | N   | 112  |
| 2971 | AAA | CTG | GCT | AAC | CTG | TTC | ATC            | GTT | AAC | AAC | AAA | AAA | ACC  | ATC | CCG | AAC | AAC | CTG | GTT | GAA | 3030 |
| 113  | K   | L   | A   | N   | L   | F   | I              | V   | N   | N   | K   | K   | T    | I   | P   | N   | N   | L   | V   | E   | 132  |
| 3031 | AAC | TAC | CTG | ACC | CCG | ATG | TCT            | CTG | GCA | TAC | TGG | TTC | ATG  | GAT | GAT | GGT | GGT | AAA | TGG | GAT | 3090 |
| 133  | N   | Y   | L   | T   | P   | M   | S              | L   | A   | Y   | W   | F   | M    | D   | D   | G   | G   | K   | W   | D   | 152  |
| 3091 | TAC | AAC | AAA | AAC | TCT | ACC | AAC            | AAA | TCG | ATC | GTA | CTG | AAC  | ACC | CAG | TCT | TTC | ACT | TTC | GAA | 3150 |
| 153  | Y   | N   | K   | N   | S   | T   | N              | K   | S   | I   | V   | L   | N    | T   | Q   | S   | F   | T   | F   | E   | 172  |
| 3151 | GAA | GTA | GAA | TAC | CTG | GTT | AAG            | GGT | CTG | CGT | AAC | AAA | TTC  | CAA | CTG | AAC | TGT | TAC | GTA | AAA | 3210 |
| 173  | E   | V   | E   | Y   | L   | V   | K              | G   | L   | R   | N   | K   | F    | Q   | L   | N   | C   | Y   | V   | K   | 192  |
| 3211 | ATC | AAC | AAA | AAC | AAA | CCG | ATC            | ATC | TAC | ATC | GAT | TCT | ATG  | TCT | TAC | CTG | ATC | TTC | TAC | AAC | 3270 |
| 193  | I   | N   | K   | N   | K   | P   | I              | I   | Y   | I   | D   | S   | M    | S   | Y   | L   | I   | F   | Y   | N   | 212  |
| 3271 | CTG | ATC | AAA | CCG | TAC | CTG | ATC            | CCG | CAG | ATG | ATG | TAC | AAA  | CTG | CCG | AAC | ACT | ATC | TCC | TCC | 3330 |
| 213  | L   | I   | K   | P   | Y   | L   | I              | P   | Q   | M   | M   | Y   | K    | L   | P   | N   | T   | I   | S   | S   | 232  |
| 3331 | GAA | ACT | TTC | CTG | AAA | TAA | (SEQ ID NO:1)  |     |     |     |     |     |      |     |     |     |     |     |     |     |      |
| 233  | E   | T   | F   | L   | K   | *   | (SEQ ID NO:2). |     |     |     |     |     |      |     |     |     |     |     |     |     |      |

LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, DC 20005  
202-408-4000

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Figs. 10A and 10B show the nucleotide sequence and restriction sites of regions of the plasmid pAF100 (SEQ ID NOS: 45-50).

The enzyme I-SceI has a known recognition site. (ref. 14.) The recognition site of I-SceI is a non-symmetrical sequence that extends over 18 bp as determined by systematic mutational analysis. The sequence reads: (arrows indicate cuts)

↓

|    |                    |                            |
|----|--------------------|----------------------------|
| 5' | TAGGGATAACAGGGTAAT | 3' ( <u>SEQ ID NO:51</u> ) |
| 3' | ATCCCTATTGTCCCATTA | 5' ( <u>SEQ ID NO:52</u> ) |

4b

On pages 41 to 42, the bridging paragraph has been amended as follows:

-e- The supernatant of this clone was used to infect other mouse cells (1009) by spreading  $10^5$  virus particles on  $10^5$  cells in DMEM medium with 10% fetal calf serum and 5 mg/ml of "[polybrain] polybrene (hexadimethrine bromide)". Medium was replaced 6 hours after infection by the same fresh medium.

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LAW OFFICES

FINNEGAN, HENDERSON,  
FARABOW, GARRETT,  
& DUNNER, L.L.P.  
1300 I STREET, N. W.  
WASHINGTON, DC 20005  
202-408-4000

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